

How Variables Like Parent, Seed Size and Desiccation Time Affect Seed Deterioration of *Quercus macrocarpa* L.

Valasia Iakovoglou*, Richard B. Hall, Allen D. Knapp,
Manjit K. Misra and Susan A. Duvick

ABSTRACT

Desiccation sensitivity is the main characteristic of recalcitrant seeds that poses limitations to practices such as storage and direct seeding. This study investigated the effect of parent tree, seed size, and desiccation time on seed variables, seedling growth, and starch thermal properties for *Quercus macrocarpa*. We expected to find an effect of parent tree, but not for the seed size due to narrow size ranges available for the study. We hypothesized that desiccation time would negatively affect seed variables and seedling growth, while starch thermal properties would also be altered. Two parent trees with distinctive pericarp characteristics (Type-1 with tougher/darker pericarp than Type-2), and two seed sizes (19.5 and 20.5 mm in diameter) for each parent were selected. Seeds were placed to desiccate in a room at ambient relative humidity, and temperature $> 21^{\circ}\text{C}$, with seeds being sampled every two days. Parent tree and seed size affected almost all seed variables and the onset of starch gelatinization, with larger values for larger seeds. Parent tree also affected seedling growth, with larger values for the Type-1. Desiccation time negatively affected the majority of the studied variables. Respiration decreased with desiccation time, with greater values for larger seeds. Our results suggest that practices such as direct seeding could be enhanced by proper parent tree and seed size selection, since those variables could delay the deleterious effects of desiccation. Starch thermal properties also proved to be indicators in tracking metabolic alterations during desiccation.

INTRODUCTION

Recalcitrant seed behavior poses limitations to practices such as direct seeding, nursery storage, and germplasm conservation (Pence, 1992) for high value species like *Quercus* spp. (Olson, 1974). *Q. macrocarpa* L. (bur oak) belongs to the *Leucobalanus* subgenus (white oaks) and is a high value species, not only due to its exceptional wood quality and aesthetic value, but also due to its great importance as mast production tree. Its recalcitrant seeds do not undergo maturation drying, maintaining hydrated condition at shedding time that loses viability upon drying (Roberts, 1973; Olson, 1974). The unique seed physiology of bur oak combines drying sensitivity with chilling tolerance (plus or minus

Valasia Iakovoglou* and Richard B. Hall, Department of Natural Resources Ecology and Management, Iowa State University, 339 Science II, Ames, IA 50011-1021; Manjit K. Misra, Seed Science Center, Iowa State University, 102 Seed Science Center, Ames, IA 50011-3228; Allen D. Knapp, Department of Agronomy, Iowa State University, Ames, IA 50011-1010; Susan A. Duvick, USDA ARS, Iowa State University, Ames, IA 50001-1010. *Corresponding author (E-mail: viakovoglou@yahoo.com). Received 29 November 2007.

close to 0 °C), and a type of dormancy that requires cold stratification for 30 to 60 days (Olson, 1974; Bonner and Vozzo, 1987), making this species of particular research interest. Its primary storage seed reserve is starch (45.9%) that is accompanied by low lipid levels (4.8%), based on whole seed measurements (Bonner and Vozzo, 1987). The effect of desiccation has been addressed for a number of variables for the recalcitrant species of *Quercus* spp. (Bonner, 1996; Connor et al., 1996; Connor and Sowa, 2003), but no research has been conducted on bur oak that also investigates the effect of parent tree and seed size.

Research has addressed the effects of desiccation based on slow/fast drying on percent germination and electrolyte leakage for a number of recalcitrant species (Farrant et al., 1985; Farrant et al., 1993; Bonner, 1996; Pammenter et al., 1998; Liang and Sun, 2000; Wesley-Smith et al., 2001). Independently of the drying rate, it has been suggested that as desiccation progressed, there was a reduction in seed viability and an increase in cell damage as reflected by higher levels of electrolyte leakage (Farrant et al., 1985; Farrant et al., 1993; Bonner, 1996; Pammenter et al., 1998; Liang and Sun, 2000; Wesley-Smith et al., 2001). Drying exposes the seeds to deleterious reactions, including increased levels of free radicals resulting from unregulated metabolism at certain moisture levels and eventually leading to loss of seed viability (Chaitanya and Naithani, 1994; Li and Sun, 1999; Greggains et al., 2001). It has also been indicated that respiration rates, declined as desiccation progressed (Finch-Savage et al., 1993; Lin and Chen, 1995; Walters et al., 2001). Leprince et al. (1999) noted in seeds of *Castanea sativa* Mill. that the cotyledon and the embryo axis presented contrasting reduced respiration patterns as desiccation progressed. Oxygen uptake of cotyledons initially increased during drying from 66 to 50% moisture content but declined upon further drying, while the embryo axes had a continuous, non-linear decline in respiration.

The negative effect of desiccation has been addressed on seedling growth by researchers such as Bhattacharyya and Basu (1992) who noted a reduction on root and shoot length of 35-day old seedlings for the recalcitrant species of jackfruit (*Artocarpus heterophyllus* Lam.). Raja et al. (2001) showed similar trends in root and epicotyl length for avocado (*Persea Americana* Mill.) seeds during desiccation. The recalcitrant species of cocoa (*Theobroma cacao* L.) subjected to four days of drying had reduced seedling growth, growth rate, and germination, accompanied by an increase in electrolyte leakage (Toruan et al., 2000).

Research has also addressed alteration of carbohydrate levels during desiccation. Toruan et al. (2000) found that sucrose, raffinose, and arabinose levels increased as desiccation progressed for cocoa (*Theobroma cacao*) species. Connor and Sowa (2003) found that sucrose levels in the embryo axis and cotyledon tissue of *Q. alba* L. seeds increased when they were exposed to dehydration stress. As desiccation progressed, the axis retained greater amounts of sucrose compared to cotyledon tissue that served as protective substrate against cell wall damage, but it did not prevent viability loss. Lin and Huang (1994) who studied the effect of desiccation on recalcitrant seeds of 16 tree species, concluded that the ratio of oligosaccharides to sucrose was not the reason for the short life-span of recalcitrant species. The above researchers addressed

metabolic changes during desiccation based on quantitative alterations of simple carbohydrates (e.g. sucrose). Those simple carbohydrates were the result of the break down of more complex storage forms, such as starch.

Many species, such as bur oak, have starch as the main form of storage carbohydrate (Bonner and Vozzo, 1987). The quality of starch can be analyzed by thermal analysis of heat flow profiles (thermograms) that can be characterized by their position (e.g. onset and peak temperatures), shape (wider bases related with a wide range of temperatures), and size (based on energy of the reaction). These profiles quantify thermal properties of starch such as gelatinization that is defined as the collapse of molecular orders, where amylose tends to leach from the starch granule, while amylopectin becomes hydrated (Whistler and Daniel, 1984). Different starch quality behaves differently during gelatinization. For example, larger granules require longer time to complete gelatinization (Koch and Jane, 2000). The genetic origin could also affect starch quality as was indicated by White et al. (1990) among and within corn populations. Ng et al. (1997) also indicated a genetic impact on starch quality when compared for waxy corn mutants that contained less than 1% amylose compared to regular corn (~25% amylose). Alteration in starch quality due to metabolic activities can also be addressed by thermal properties as Ng et al. (1997) indicated those changes by using starch thermal analysis during developmental stages of corn. Gamel et al. (2005) also indicated starch alterations based on thermal analysis results when compared germinated amaranth seedlings to non-germinated. No research has used starch thermal analysis to address any variation in starch quality based on the genetic effect of parent tree and seed size for any of the forestry species. Further, no research has used starch thermal properties as indicators on the onset of biochemical alterations during desiccation for recalcitrant seed species, or any species at all.

The difficulties that forestry species pose in making controlled pollinations compared to agricultural crops (Martinelli and Carvalho, 1999; Johnson et al., 2001; Alt et al., 2002) combined with their late maturation age (> 20 years old to produce seeds, Olson, 1974) and their sporadic acorn production, make the study of parental effects rather difficult. To our knowledge, limited research has addressed the parental effect on seedling growth (Kormanik et al., 1998; Iakovoglou et al., 2007), with no research studying the potential effect of parent tree, seed size, and desiccation time on seed variables, seedling growth, and starch thermal properties on any forestry species. Although research has addressed changes in physical and biochemical characteristics of recalcitrant seeds during desiccation, more research on additional species could assist on understanding how other recalcitrant species with unique characteristics such as bur oak behave under desiccation. More research could also assist in identifying variables such as starch thermal properties that could be used as sensitive indicators on the onset of seed degradation during desiccation.

In our study, we hypothesized that there should be an effect of parent tree that reflects the importance of genetic background of selecting specific parent trees. We did not expect to find a seed size effect because of the narrow range of sizes available for the study. Finally, we wanted to investigate how all these

variables change as desiccation progressed, and if some of these variables are more sensitive indicators of desiccation than the moisture content of the seed. Also, we postulated that starch thermal analysis might be a sensitive indicator of qualitative changes in starch as seed progress through desiccation.

Studying alterations of the physical and biochemical properties of the recalcitrant species such as bur oak during desiccation might provide insights about the nature of recalcitrant behavior, and how variables like parent and seed size might affect seed deterioration. Any information leading to developments that would delay seed deterioration could improve storage and the success of direct seeding, thereby increasing the use of these species. Starch thermal analysis may prove to be a useful technique allowing early detection of qualitative starch alterations that can be used as mark points to study metabolic alterations in seeds.

MATERIALS AND METHODS

Seed collection

Seeds were collected under the crown of open grown trees located in Ames (IA) (42° 03' latitude, and 93° 04' longitude), in September 2001. At least ten trees were closely monitored until the time of seed shedding. Due to their sporadic seed production (every 3 to 5 years) (Sork et al., 1993; Greenberg and Parresol, 2000) we were able to get adequate seeds for the study only from two parent trees that had distinct pericarp seed characteristics. Based on toughness and color, the parent tree with the tougher (thicker), darker pericarp was referred to as the "Type-1" parent, while the other parent tree with thinner and lighter pericarp color was labeled as the "Type-2" parent. The mean fresh pericarp weight (of 8 seeds) of Type-1 was approximately 0.18 g heavier than the Type-2 parent (1.15 vs 0.97 g) (Iakovoglou, 2005). A high frequency of double embryos was another noticeable characteristic for the Type-2 parent tree. Those double embryos were discarded. To guarantee proper seed maturation, collections were made from the ground on a daily basis until the majority of the seeds were shed. After each day of collection, seeds were floated overnight to discard the majority of damaged and weevil infested seeds (Bonner and Vozzo, 1987; Gribko and Jones, 1995). This treatment results in some moisture uptake to recover any seed moisture lost from dehydration that might have occurred from shed prior to seed collection (Bonner and Vozzo, 1987). Following the floating treatment, the attached seed-cap was removed with a knife. Each seed was carefully checked for weevil or any other type of defects. Seeds were stratified prior to laboratory tests in polyethylene bags (Gosling, 1989) and stored in a walk-in cooler at 4 ± 1 °C for 60 d.

Seed diameters were measured with digital calipers to establish two similar seed size ranges of 19–20 (19.5), and 20–21 (20.5) mm for each parent tree while providing enough seed from each source to conduct our experiments. Seeds were placed in a controlled environment room, with ambient humidity levels and temperatures not dropping below 21 °C to allow slow drying of the seeds. Seeds were sampled, on two-day intervals for a total of two weeks to determine the effect of desiccation on a number of seed variables, seedling growth and starch thermal properties.

Seed variables

A set of four randomly selected seeds of each seed size from each parent tree was taken for each sampling time. Seed moisture content on a fresh weight basis (MC) = [(seed fresh weight - seed dry weight)/(seed fresh weight)] * 100, seed fresh weight (SFW), pericarp fresh weight (PFW), embryo fresh (EFW) and embryo dry weight (EDW) were measured at each sampling time. Seed and embryo dry weights were determined by placing them in aluminum pans and drying in a convection oven at 103 °C for 17 h, according to proper procedures for large and high moisture content seeds (Bonner, 1981; ISTA, 1999).

Each sampling time, an additional random sample of two seeds was used to evaluate respiration using a Gilson Differential Respirometer (Gilson Medical Electronics, INC., Middleton, WI, USA) (Umbreit et al., 1964). Each seed was split by removing one cotyledon so that it could fit into the respirometer chamber. The remaining cotyledon was trimmed around the embryo axis to give an attached segment of cotyledon that was approximately 1 cm in length by 1 cm wide to properly fit in the small vials used by the Gilson Respirometer without damaging the embryo axis. Respiration was monitored for each seed for a period of 3 min every 15 min for a total of 2 h. After respiration measurements, the embryo tissue (embryo axis plus cotyledon part) was oven dried (ISTA, 1999). The reported respiration rates are based on μL of O_2 uptake per minute per gram dry matter ($\mu\text{L O}_2 \text{ min}^{-1} \text{ gr}^{-1}$).

Seedling growth

For each sampling time, ten seeds were randomly selected for each seed size of each parent tree to determine the percent of germination and seedling growth. Seeds were placed on trays that contained moist paper (Kimpak), and grown for 4 weeks in growth chamber under a diurnal cycle of 16 h of dark at 20 °C altered with 8 h of light at 30 °C (ISTA, 1999). At the end of the fourth week, seedling growth was assessed by measuring maximum root width directly below the root collar, root length, and epicotyl length. All measurements were made in millimeters using digital calipers.

Starch thermal properties-seedling growth

For each seed size of each parent tree, a total of five seeds were randomly selected to determine starch characteristics. For each seed, the distal 1/3 of the cotyledons was removed, with one cotyledon part being used for starch analysis. The rest of the embryo (embryonic axis plus the 2/3 of the cotyledons) was studied for seedling growth, similarly as described above (ISTA, 1999). Seedling growth was assessed at the fourth week by measuring maximum root width (mm), root length (mm), and epicotyl length (mm).

Starch extraction was done as described by White et al. (1990). The cotyledon tissue was placed in a tube with distilled water and homogenized using a POLYTRON PT3100 (Kinematica AG, Luzernerstrasse 147a, CH-6014 Littau Luzern, Switzerland) laboratory homogenizer equipped with a POLYTRON PT-DA 3012/2T generator. Homogenized cotyledons were screened through nylon mesh with mesh opening: 30 μm , percent open area: 21, and thickness:

64 μm (Spectramesh®Spectrum Laboratories Inc. Rancho Dominguez, CA, USA) the starch granules passed through the mesh into a collection flasks and the residue (protein, fiber and starch aggregates) retained by the screen was discarded. Then the starch slurry was transferred to 250 mL beakers and the starch granules allowed settling for two hours at 4 °C in a refrigerator. The upper phase was then decanted leaving a starchy substrate at the base of the beaker. The remaining starch was re-suspended in distilled water and allowed to settle for one hour. This wash-settle-decant process was repeated three times, and the remaining starch was air dried at room temperature with a fan circulating the air. The dried starch samples were scraped out of the beakers and stored in glass vials.

A differential scanning calorimeter (DSC) thermal analysis was accomplished by using a Perkin-Elmer DSC-7 attached with a thermal analyzer station (Perkin-Elmer Co., CT). Approximately 3.5 mg of the starch sample was weighed in an aluminum pan and 8 μL of distilled water was added before hermitically sealing the pans with a crimper press. The samples were equilibrated at room temperature for 2 h prior to analysis. To measure the gelatinization events, samples were heated in the DSC from 300 to 110 °C with a heating rate of 10 °C per minute. Data recorded from the thermal curve included: onset of gelatinization (T_{OG}) in °C, peak temperate of gelatinization (T_{PG}) in °C, and enthalpy (ΔHG) in J/g. The calculated values include: range of gelatinization $\text{RnG} = 2(T_{\text{PG}} - T_{\text{OG}})$, peak height index (PHI) as $\Delta\text{HG}/(T_{\text{PG}} - T_{\text{OG}})$.

Statistical analysis

The data were modeled with a linear model and parent, seed size, desiccation time, and their interactions were considered fixed effects (SAS Institute Inc., 2002). Individual comparisons between effects of interest were conducted with *t*-tests. Analyses were done with the GLM procedure of SAS® V9.0 (2002). Pearson correlations were computed to determine correlations among the studied variables.

RESULTS

Parent tree and seed size effects were evident for most of the studied variables; seed variables, seedling growth, and starch thermal properties. Sampling time, reflecting the desiccation level of the seeds, was negatively correlated with the majority of the variables studied.

Seed variables

The effect of parent tree was significant for all seed variables, except respiration (Table 1). Seed size affected all variables studied with larger mean values for larger seed size. Desiccation time affected all seed variables, except the embryo dry weight. There was an interaction effect of parent tree * time for the respiration.

Based on multi-correlation comparisons when combining all seed sizes from both parent trees, there was a negative correlation between desiccation time and most of the seed variables (Fig. 1).

TABLE 1. The level of significance based on linear models of bur oak parent tree (P), seed size (S), time of desiccation (T), and all possible interactions on seed variables, described by seed- (SFW), pericarp- (PFW), embryo- fresh (EFW) and dry weight (EDW), moisture content (MC), and respiration (R), as well as four week-old seedling growth described by root width (RW), root length (RL), and epicotyl length (EL).

Source	DF	Level of significance								
		Seed variables						Seedling growth		
		SFW	PFW	EFW	EDW	MC	R	RL	RW	EL
Parent (P)	1	<.0001	<.0001	0.0078	0.0009	0.0168	0.7612	<.0001	0.0003	0.1461
Seed size (S)	1	<.0001	<.0001	<.0001	<.0001	0.0413	0.0010	0.2877	0.6933	0.7996
Time (T)	7	<.0001	<.0001	<.0001	0.3702	<.0001	0.0007	<.0001	0.0053	<.0001
P*S	1	0.7990	0.7314	0.6817	0.8656	0.3240	0.5944	0.1970	0.4045	0.4557
P*T	7	0.2434	0.5956	0.4830	0.8286	0.1393	0.0260	0.4116	0.8219	0.3248
S*T	7	0.0876	0.6848	0.1269	0.7642	0.6077	0.3961	0.1119	0.9547	0.5177
P*S*T	6	0.6520	0.5367	0.8261	0.8996	0.1585	0.2746	0.4862	0.0578	0.6981

Seedling growth

Percent germination was negatively correlated with desiccation time ($r = -0.66$, p -value = < 0.0001). A substantial reduction in germination began after approximately six days of desiccation. The germination declined from ~100 to 60% on the 14th day of the experiment (Fig. 1). An effect of parent tree for four-week-old seedlings was evident for the root width and length, with larger mean values for the Type-1 parent for the root length (194.22 versus 147.91 mm) and the root width (2.79 versus 2.51 mm). Seed size did not affect seedling growth for any variables studied (Table 1). Time of desiccation affected all variables related to seedling growth. Root width ($r = -0.2$, p -value = 0.0008), root length ($r = -0.32$, p -value = < 0.0001), and epicotyl length ($r = -0.4$, p -value = < 0.0001) were negatively correlated with desiccation time (Fig. 1).

Starch thermal properties-seedling growth

A graphic presentation of the endothermic starch curves for desiccation time 0 d (thick line) and 14 d (thin line) is provided by Fig. 2. Parent tree affected gelatinization onset (T_0G) and the peak height index (PHI), with smaller mean values for the Type-1 parent (55.89 °C and 0.52, respectively) compared to the Type-2 parent (56.80 °C and 0.58, respectively) (Table 2). The range (RnG) and change in enthalpy (ΔHG) values were larger for the Type-1 parent (14 °C and 13.32 J/g, respectively) than for the Type-2 parent (11.58 °C and 12.50 J/g, respectively). Seed size and desiccation time affected T_0G , with a larger mean value for larger seeds (20–21 mm) (56.84 versus 55.85). Alternatively, the ΔHG values were larger for smaller seeds (19–20 mm) (13.18 versus 12.65). Desiccation time also affected the peak temperature of gelatinization

(T_pG) with a reduction in the mean value as time progressed. There was an interaction effect for parent tree * seed size, and parent tree * seed size * time for the ΔHG , and a parent tree * time interaction for the T_0G . Desiccation time was negatively correlated with T_0G ($r = -0.41$, p -value = 0.001), T_pG ($r = -0.4$, p -value = 0.002), and PHI ($r = -0.29$, p -value = 0.022) (Fig. 3).

Seed from the Type-1 parent tree produced longer roots at four weeks (194.01 mm) than the Type-2 parent (138.24 mm) (Iakovoglou, 2005). The

FIGURE 1. The effect of desiccation time on bur oak seed variables (seed-, pericarp-, embryo-fresh weight, embryo dry weight, moisture content, respiration, and percent germination), and seedling growth at four weeks (root width, root length, and epicotyl length). Correlation values are also provided (p -values in parenthesis); NS refers to non-significant correlations at $p < 0.05$.

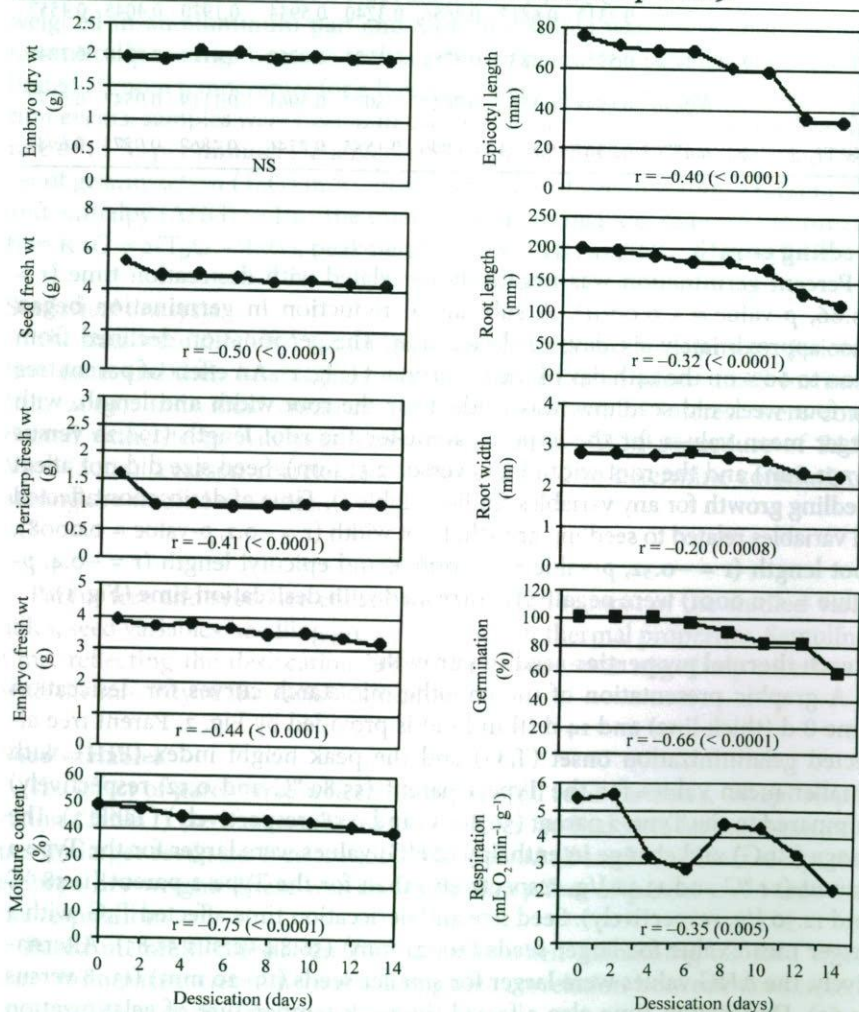


FIGURE 2. A graphic presentation of endothermic starch curves at time 0 d of desiccation (thick line) and 14 d of desiccation (thin line) that indicates a shift of the onset and the peak of starch gelatinization temperatures during desiccation. The lines presented are for two bur oak acorns of the same size from the same parent tree (Type-1, with rougher/thicker pericarp) that were representative of the general trends observed.

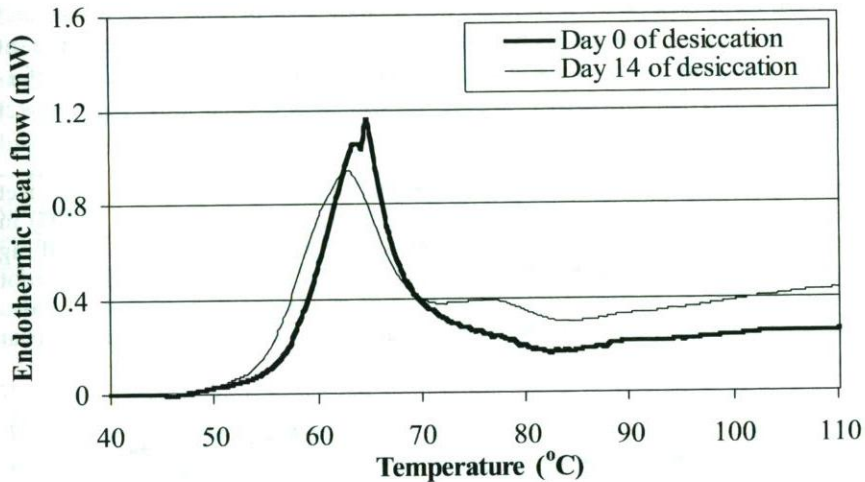


TABLE 2. The level of significance based on linear models of bur oak parent tree (P), seed size (S), time of desiccation (T), and all possible interactions on seed starch thermal properties described by onset (T_{0G}), peak temperature (T_{pG}), enthalpy (ΔHG), range (RnG), and peak height index (PHI) of gelatinization, and seedling growth as described by root width (RW), root length (RL), and epicotyl length (EL) for four week-old seedlings derived from 2/3 of the seed.

Source	DF	Level of significance							
		Starch thermal variables					Seedling growth (2/3 of seed)		
		T_{0G}	T_{pG}	HG	RnG	PHI	RL	RW	EL
Parent (P)	1	0.0096	0.2526	0.0006	<.0001	0.0010	0.0018	0.0819	0.9445
Seed size (S)	1	0.0051	0.1590	0.0172	0.0589	0.2059	0.5459	0.2521	0.2835
Time (T)	7	0.0010	0.0151	0.0017	0.2501	0.0635	0.0527	0.7250	0.0986
P*S	1	0.4140	0.2849	0.0261	0.8159	0.1510	0.0930	0.3900	0.7531
P*T	7	0.0397	0.3745	0.0847	0.5462	0.6702	0.5563	0.3653	0.8925
S*T	7	0.2139	0.6057	0.2956	0.3482	0.0701	0.0452	0.0895	0.0319
P*S*T	6	0.1716	0.2136	0.0349	0.8323	0.5285	0.6628	0.9936	0.5933

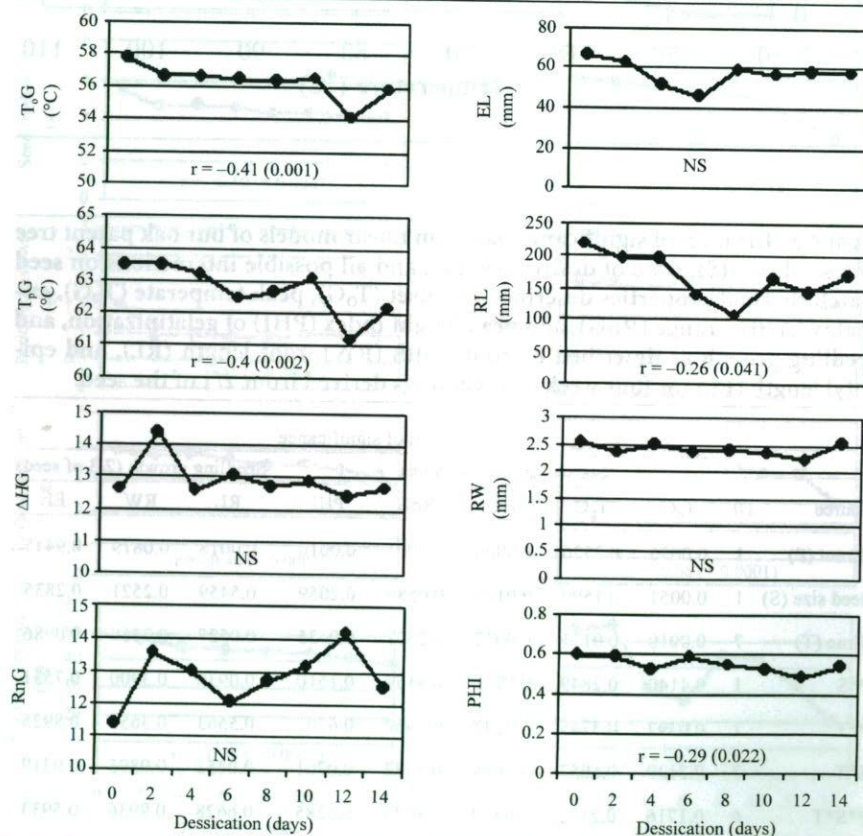
interactions between size and time for the root and epicotyl length were significant (Table 2). A negative correlation with desiccation time was evident only for the root length (Fig. 3).

DISCUSSION

Parent tree

In spite of the thin pericarp of the Type-2 parent, there was little difference in the dry down patterns of the seed between the two parents. Despite the fact that after seed collection all seeds were treated consistently the same, different seed maturation levels at shedding time might have also resulted in differences between the sampled trees. Levels of moisture content might also reflect

FIGURE 3. The effect of desiccation time on starch thermal properties [onset (T_{0G}) and peak temperature of gelatinization (T_{pG}) in $^{\circ}\text{C}$, enthalpy (ΔH_G) in J/g, range of gelatinization (RnG), and peak height index (PHI)], and seedling growth at four weeks (derived from $2/3$ of the seed) [root width (RW), root length (RL), and epicotyl length (EL)] for bur oak. Correlation values are also provided (p -values in parenthesis); NS refers to non-significant correlations at $p < 0.05$.



variation in maturation levels of the seeds. Based on our data, the mean initial moisture content between parent trees indicated an approximate difference of 4% in moisture content (46.8% for the Type-1 parent, and 51.1% for the Type-2 parent). Difference in moisture level between the two parent trees might have been the reason why there was an interaction effect of parent tree and desiccation time on seed respiration (Table 1). The effect of parent tree was also indicated at the seedling growth, as reflected by the root width, root length, and epicotyl length, with larger mean values for the Type-1 parent. That suggests that the effect of parent tree carries on beyond the seed level, indicating the importance in selecting a number of specific parent trees as a genetic source for seeding sites that face regeneration problems, since more vigorous seedling growth should enhance establishment success.

Parent tree affected starch thermal properties indicating qualitative starch differences among seeds from different sources, with lower onset and larger enthalpy mean values for the Type-1 parent. In addition to the low number of parent trees used in this study, those differences could also be affected by variation in seed maturation levels among parent trees. Ng et al. (1997), who studied starch thermal properties at developmental stages of a number of maize (*Zea mays* L.) mutants, also suggested that thermal properties differ based on the genetic background and developmental stage of the seed. White et al. (1990) also indicated differences in starch thermal properties among and within maize populations, emphasizing the importance of genetic origin on the starch quality of food reserves. Our research presents differences in starch quality based on selection of specific parent tree on recalcitrant forestry species. Most research that has been done on seedling growth and biochemical alterations of recalcitrant forestry species was based on blocked seeds pooled together from a number of parent trees (Bonner and Vozzo, 1987; Bonner, 1996; Connor et al., 1996; Connor and Sowa, 2003). The lack of research on the parental effect particularly on biochemical properties such as starch thermal properties underlines the need for more research. Further studies on a greater number of parents should assist in increasing our knowledge on the parental effect and understanding of its effect at a biochemical level.

Seed size

Despite the fact that the two seed sizes used in this research differed by only 1 mm in diameter, there was an effect of size for seed variables with larger values for larger seeds (20.5 mm in diameter). Specifically for moisture content that might suggest that smaller seeds might tend to retain less moisture during desiccation than the larger seeds. That has also been observed by Daws et al. (2004) for the recalcitrant species of *Vitellaria paradoxa* Gaerther F., with smaller seeds (4–6 g based on fresh seed mass) drying faster compared to larger (8–10 g). Chacón and Bustamante (2001) also noted an effect of seed size during desiccation for the recalcitrant species of *Cryptocarya alba* Moll., with smaller seeds (< 0.61 g) drying faster than larger seeds (> 1.32 g). Specifically for recalcitrant species, the ability of larger seeds to dry slower might benefit regeneration and storage practices by decreasing mortality rates. Based on our

results, seed metabolism as reflected by respiration values was also higher for the larger seed size ($4.7 \mu\text{L of O}_2 \text{ min}^{-1} \text{ g}^{-1}$) compared to the smaller ($3.4 \mu\text{L of O}_2 \text{ min}^{-1} \text{ g}^{-1}$). That could be the result of the ability of larger seeds to retain greater moisture content during desiccation.

Quantitative analysis of qualitative starch thermal properties also indicated seed size differences, with higher onset temperatures for the larger seeds, and greater enthalpy for the smaller seeds. That suggests that size is a potential trait to facilitate selection based on starch thermal properties. That information could also be useful to the "cooking" industry for other agronomic species, since starch thermal analysis has been mainly used to study starch-cooking properties. By simply using an easy trait, such as "seed size", we could also pre-select for complex biochemical traits, such as starch thermal properties. However, it needs to be mentioned that the effect of seed size did not have a carry-over on seedling growth at four weeks, indicating that those variables might be more related to seed characteristics independent of seed size.

Desiccation time

Desiccation time seemed to have a negative effect on almost all studied variables. Among the seed variables, only the embryo (embryo axis plus cotyledon tissue) dry weight did not indicate any quantitative alterations during desiccation. During the first two days of desiccation, there was substantial reduction in the seed fresh weight (pericarp plus embryo) that could be attributed to the abrupt reduction in pericarp fresh weight rather than reduction of the embryo fresh weight. That might suggest that although the pericarp reduces desiccation stress, its protective abilities might be only for a few days. Sobrino-Vesperinas and Viviani (2000) who studied the morphology of the pericarp for *Q. suber* L. suggested that it plays a protective role to desiccation during seed development, but after the seed is shed, the pericarp serves more like a water absorbing seed-structure.

Respiration also decreased as time of desiccation progressed. Walters et al. (2001) noted for embryo axis tissue of tea (*Camellia sinensis* (L.) Kuntze) a reduction in respiration during desiccation. In our results, although reduction occurred, there was a boost in respiration at the eighth day of drying, then, after that point respiration started to decrease again. The unaccounted variation due to the small sample size used for respiration measurements could have affected the observed results. However, the fact that respiration measurements were based on seed tissue that retained both embryo axes and the proximal 1 cm^2 of cotyledon tissue might have also resulted in the respiration boost during the eighth day of desiccation. Leprince et al. (1999) indicated for *Castanea sativa* Mill. that embryo axes and the cotyledon tissue exhibited contrasting respiration patterns during desiccation. Based on their results, O_2 uptake by the embryo axis was gradually declining until the seed water content reached values as low as $1.3 \text{ g H}_2\text{O/g dry weight}$, while cotyledons during that time showed a burst in respiration. Based on their measurements, after that point, respiration levels of both cotyledon and embryo axis tissue gradually declined. So based on our measurements on the eighth day of desiccation, seeds might

have been at that point where, although respiration decreases as desiccation progresses, there is a burst in respiration most likely due to the cotyledon tissue that surrounds the embryo axis that was used to quantify respiration.

The reduction in respiration during the sixth day of desiccation coincided with the start of the reduction in percent germination. That suggests that bur oak can sustain desiccation for almost a week for seeds that had their cap removed and were exposed to a laboratory environment that might well differ from natural conditions. After that period of time, the negative impact of desiccation was evident in the percent of germination. That was further indicated by seedling growth at four weeks, where root and epicotyl length were substantially reduced after the sixth day of desiccation. However, reduction in seedling growth measurements following seed desiccation occurred even after the first two days of desiccation, suggesting that seedling growth variables might be a more sensitive indicator than percent germination in studying adverse effects of desiccation on regeneration success. Bhattacharyya and Basu (1992) also noted reduction in seedling growth for the recalcitrant species of jackfruit (*Artocarpus heterophyllus* Lam.) based on 35-day-growth of root and shoot length. For *T. cacao*, Toruan et al. (2000) showed a reduction in seedling growth rate and seedling dry weight when exposed to four days of drying. Raja et al. (2001) noted similar trends during desiccation in seedling growth as reflected by root and epicotyl length for avocado (*Persea Americana* Mill.) seeds.

Time of desiccation also seemed to have an effect on starch thermal properties, resulting in a change of the shape of the endothermic curve to lower onset and peak gelatinization temperatures that could be an indication of starch degradation during desiccation. Ng et al. (1997), who studied starch thermal properties during developmental stages for a number of maize (*Zea mays* L.) mutants, suggested that alterations in starch thermal values could have been attributed to alteration in amylose content and other materials associated with the starch grains. By using starch thermal properties, Gamel et al. (2005) also showed that germinated seeds (dried at 90 °C) had greater gelatinization energy compared to raw seeds, while there was also a reduction in the starch quantity for the germinated seeds. That suggests that starch thermal properties might be a sensitive variable that indicates biochemical changes during metabolic alterations such as developmental (Ng et al., 1997) and germinated seed stages (Gamel et al., 2005). To our knowledge, limited research has addressed any alterations of starch thermal properties during desiccation for any of the forestry or agricultural species. Our results showed that those properties altered during seed desiccation for the recalcitrant species of bur oak. Nevertheless, more research needs to be done on greater number of replications to better describe and address how variables such as seed size can interrelate with starch thermal properties during desiccation. Seedling growth as revealed by the remaining 2/3 of the seed-tissue, indicated a negative effect of desiccation time only for the root width. The lack of significant negative trends during desiccation could have been attributed to the great variation of the unaccounted due to the low number of replications. A greater number of parents combined with a wider seed size range could greatly aid in the findings of this

study that could be the starting point for future research. Also, the absence of pericarp of those samples compared to the germinated seeds that retained their pericarp, probably resulted in more rapid water uptake when seeds were placed in the germination chamber, leading to faster recovery from the desiccation.

Our research suggests that the deleterious effects of desiccation are evident for the majority of the studied variables. However, careful parent tree and/or seed size selection might reduce the intensity of the desiccation effect. Starch thermal properties indicated alterations during desiccation. Further study of those starch variables in relation to quantitative amylose and amylopectin content as well levels of simple carbohydrates, could also mark levels of seed metabolism. That could help understand and quantify metabolic transactions that occur prior to loss of viability when recalcitrant seeds are exposed to desiccation. Researchers could benefit by breaking barriers and expending their knowledge by using those techniques (starch thermal properties) to a wider spectrum of both agricultural and forestry species.

REFERENCES

- Alt, B.J., W.R. Fehr and G.A. Welke. 2002. Selection for large seed and high protein in two- and three-parent soybean populations. *Crop Sci.* 42:1876–1881.
- Bhattacharyya, A.K. and R.N. Basu. 1992. Retention of vigor and viability of jackfruit (*Artocarpus heterophyllus* Lam.) seed. *Indian Agr.* 36:65–74.
- Bonner, F.T. 1981. Measurement and management of tree seed moisture. USDA For. Serv. Res. Pap. SO-177, 11 p.
- Bonner, F.T. 1996. Responses to drying of recalcitrant seeds of *Quercus nigra* L. *Ann. Bot.* 78:181–187.
- Bonner, F.T. and J.A. Vozzo. 1987. Seed biology and technology of *Quercus*. USDA For. Serv. Gen. Tech. Rep. SO-66, 21 p.
- Chacón, P. and R.O. Bustamante. 2001. The effects of seed size and pericarp on seedling recruitment and biomass in *Cryptocarya alba* (Lauraceae) under two contrasting moisture regimes. *Plant Ecol.* 152:137–144.
- Chaitanya, K.S.K. and S.C. Naithani. 1994. Role of superoxide, lipid peroxidation and superoxide dismutase in membrane perturbation during loss of viability in seeds of *Shorea robusta* Gaertn. *New Phytol.* 126:623–627.
- Connor, K.F., F.T. Bonner and J.A. Vozzo. 1996. Effects of desiccation on temperate recalcitrant seeds: differential scanning calorimetry, gas chromatography, electron microscopy, and moisture studies on *Quercus nigra* and *Quercus alba*. *Can. J. For. Res.* 26:1813–1821.
- Connor, K.F. and S. Sowa. 2003. Effects of desiccation on the physiology and biochemistry of *Quercus alba* acorns. *Tree Physiol.* 23:1147–1152.
- Daws, M.I., C.S. Gaméné, S.M. Glidewell and H.W. Pritchard. 2004. Seed mass variation potentially masks a single critical water content in recalcitrant seeds. *Seed Sci. Res.* 14:185–195.
- Farrant, J.M., P. Berjak and N.W. Pammenter. 1985. The effect of drying rate on viability retention of recalcitrant propagules of *Avicennia marina*. *S. Afr. J. Bot.* 51:432–438.
- Farrant, J.M., P. Berjak and N.W. Pammenter. 1993. Studies on the development of the desiccation-sensitive (recalcitrant) seeds of *Avicennia marina* (Forssk.) Vierh.: The acquisition of germinability and response to storage and dehydration. *Ann. Bot.* 71:405–410.

- Finch-Savage, W.E., R.I. Grange, G.A.F. Hendry and N.M. Atherton. 1993. Embryo water status and loss of viability during desiccation in the recalcitrant seed species *Quercus robur* L. In Basic and Applied Aspects of Seed Biology. Proc. Fifth Intl. Workshop on Seeds, Reading. R.H. Ellis, M. Black, A.J. Murdoch and T.D. Hong (ed.). Kluwer Academic Publishers, London, pp 723-730.
- Gamel, T.H., J.P. Linssen, A.S. Mesallem, A.A. Damir and L.A. Shekib. 2005. Effect of seed treatments on the chemical composition and properties of two amaranth species: starch and protein. J. Sci. Food Agric. 85:319-327
- Gosling, P.G. 1989. The effect of drying *Quercus robur* acorns to different moisture contents, followed by storage, either with or without imbibition. Forestry. 62:41-50.
- Greenberg, C.H. and B.R. Parrelsol, B.R. 2000. Acorn production characteristics of Southern Appalachian oaks: a simple method to predict within-year acorn crop size. USDA For. Serv. Res. Paper SRS-20. Asheville, NC. 14pp.
- Greggains, V., W.E. Finch-Savage, N.M. Atherton and P. Berjak. 2001. Viability loss and free radical processes during desiccation of recalcitrant *Avicennia marina* seeds. Seed Sci. Res. 11:235-242.
- Gribko, L.S. and W.E. Jones. 1995. Test of the float methods of assessing northern red oak acorn condition. Tree Planters' Notes. 46:143-147.
- Iakovoglou, V. 2005. Desiccation and Nitrous Oxide Storage Effects on the Recalcitrant Seeds of *Quercus alba* and *Quercus macrocarpa*. PhD Dissertation, Iowa State University, Ames, IA, USA.
- Iakovoglou, V., M.K. Misra, R.B. Hall and A.D. Knapp. 2007. The effect of seed size and parent tree on seed variables and seedling growth of *Quercus macrocarpa* and *Q. alba*. Seed Sci. Technol. 35:771-777.
- International Seed Testing Association (ISTA). 1999. International rules for seed testing. Seed Sci. Technol. 27 (Suppl.), 333p.
- Johnson, S.L., W.R. Fehr, G.A. Welke and S.R. Cianzio. 2001. Genetic variability for seed size of two- and three-parent soybean populations. Crop Sci. 41:1029-1033.
- Koch, K. and J.L. Jane. 2000. Morphological changes of granules of different starches by surface gelatinization with calcium chloride. Cereal Chem. 77:115-120.
- Kormanik, P.P., S.S. Sung, T.L. Kormanik, S.E. Schlarbaum and S.J. Zarnoch. 1998. Effect of acorn size on development of northern red oak 1-o seedlings. Can. J. For. Res. 28:1805-1813.
- Leprince, O., J. Buitink and F.A. Hoekstra. 1999. Axes and cotyledons of recalcitrant seeds of *Castanea sativa* Mill. exhibit contrasting responses of respiration to drying in relation to desiccation sensitivity. J. Exp. Bot. 50:1515-1524.
- Li, C. and W.Q. Sun. 1999. Desiccation sensitivity and activities of free radical scavenging enzymes in recalcitrant *Theobroma cacao* seeds. Seed Sci. Res. 9:209-217.
- Liang, Y. and W.Q. Sun. 2000. Desiccation tolerance of recalcitrant *Theobroma cacao* embryonic axes: the optimal drying rate and its physiological basis. J. Exp. Bot. 51:1911-1919.
- Lin, T. and M. Chen. 1995. Biochemical characteristics associated with the development of the desiccation-sensitive seeds of *Machilus thunbergii* Sieb. & Zucc. Ann. Bot. 76:381-387.
- Lin, T. and N.H. Huang. 1994. The relationship between carbohydrate composition of some tree seeds and their longevity. J. Exp. Bot. 45:1289-1294.
- Ng, K.Y., S.A. Duvick and P.J. White. 1997. Thermal properties of starch from selected maize (*Zea mays* L.) mutants during development. Cereal Chem. 74:288-292.

- Martinelli, A. and N.M. De Carvalho. 1999. Seed size and genotype effects on maize (*Zea mays* L.) yield under different technology levels. *Seed Sci. Technol.* 27:999–1006.
- Olson, D.F. Jr. (1974) *Quercus* L. Oak. In *Seeds of Woody Plants of the United States*. C.S. Schopmeyer (ed.). USDA Washington, DC, pp. 692–703.
- Pammenter, N.W., V. Greggains, J.I. Kioko, J. Wesley-Smith, P. Berjak and W.E. Finch Savage. 1998. Effects of differential drying rates on viability retention of recalcitrant seeds of *Ekebergia capensis*. *Seed Sci. Res.* 8:463–471.
- Pence, V.C. 1992. Desiccation and survival of *Aesculus*, *Castanea* and *Quercus* embryo axes through cryopreservation. *Cryobiology*. 29:391–399.
- Raja, K., V. Palanisamy, P. Selvaraju and K.A. Shanmugasundaram. 2001. Desiccation sensitivity of avocado (*Persea americana* Mill.) seeds. *Newsletter Project on Handling and Storage of Recalcitrant and Intermediate Tropical Forest Tree Seeds*. 8:22–24.
- Roberts, E.H. 1973. Predicting the storage life of seeds. *Seed Sci. Technol.* 1:499–514.
- SAS Institute, Inc. 2002. *SAS/STAT User's Guide*. Release 9.00 Ed. Cary, NC.
- Sobrinho-Vesperinas, E. and A.B. Viviani. 2000. Pericarp micromorphology and dehydration characteristics of *Quercus suber* L. acorns. *Seed Sci. Res.* 10:401–407.
- Sork, V.L., J. Bramble and O. Sexton. 1993. Ecology of mast-fruitletting in three species of North American deciduous oaks. *Ecology*. 74:528–541.
- Toruan, M.N., H. Rachmawati, H. Hurhaimi and T. Hutabarat. 2000. Physiological and biochemical changes in cocoa seed (*Theobroma cacao* L.) caused by desiccation. *Menara Perkebunan*. 68:20–29.
- Umbreit, W.W., R.H. Burris and J.F. Stauffer. 1964. *Manometric Techniques, a Manual Describing Methods Applicable to the Study of Tissue Metabolism*, 4th ed. Burgess Publishing Company, Minneapolis, MN.
- Walters, C., N.W. Pammenter, P. Berjak and J. Crane. 2001. Desiccation damage, accelerated ageing and respiration in desiccation tolerant and sensitive seeds. *Seed Sci. Res.* 11:135–148.
- Wesley-Smith, J., N.W. Pammenter, P. Berjak and C. Walters. 2001. The effects of two drying rates on the desiccation tolerance of embryonic axes of recalcitrant jackfruit (*Artocarpus heterophyllus* Lamk.) seeds. *Ann. Bot.* 88:653–664.
- Whistler, R.L. and J.R. Daniel. 1984. Molecular structure of starch. In *Starch Chemistry and Technology*. 2nd ed. R.L. Whistler, J.N. BeMiller and E.F. Parrish (ed.). Academic Press, Orlando, FL, pp. 153–182.
- White, P., I. Abbas, L. Pollak and L. Johnson. 1990. Intra- and interpopulation variability of thermal properties of maize starch. *Cereal Chem.* 67:70–73.